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SPECIFICATION

TITLE

"METHOD AND DEVICE TO LOCALIZE LIGHT-EMITTING REGIONS"

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention concerns a method as well as a device to implement the method to localize regions, in particular focal lesions in a biological tissue section that, at least during the examination, exhibits a fluorescence property distinct from the tissue section, due to which, given an exposure with light of a first wavelength, light of another wavelength is emitted.

Description of the Prior Art

In Umar Mahmood et al., "Near Infrared Optical Imaging of Protease Activity for Tumor Detection", Radiology 213:3, 866-870 (1999), it is specified that fluorescing metabolic markers either accumulate exclusively in specific regions, for example tumors, inflammations or other specific metastases, or are distributed throughout the body, but are activated only in specific regions, for example by tumor-specific enzyme activities and by additional exposure by means of light.

This principle of optical fluorescence imaging is explained using Figure 1, in which a tumor is visible given the exposure with NIR light (light in the near-infrared range) after a marker whose fluorescence properties are activated by specific enzymes was administered to a mouse.

The recognition of a tumor or another marked region then ensues by exposure of the region with light in the special excitation wavelengths of the fluorescent dye, and detection of the emitted light in the corresponding emission wavelength of the fluorophore. Given authorization for use on humans, these markers can be used in early cancer detection, for example.

By exciting the dye with at least one temporally varied excitation light signal, for example by temporal variation of irradiation location and/or light wavelength and/or intensity modulation of the excitation light, data can be acquired (for example by means of CCD or photomultiplier, photon flux on the surface of the tissue section, such as, for example, the female breast) at different measurement points at different variations. In this manner, variation-dependent and location-dependent – meaning spatially two-dimensional – measurement data are acquired. In the case of M measurement data of N variations, these are MxN data.

From these data, spatially delimited lesions such as, for example, focal fluorescing marked tumors with different fluorescence properties than the surrounding tissue can be located three-dimensionally.

The method is provided in cancer (screening) examinations of the breast, lymph nodes, thyroid, prostate, and has intraoperative applications, and in general is applicable for all organs near the surface that lie in the range of the penetration of light and that develop carcinoma (or other diseases) for which (now or at a future point in time) corresponding fluorescent markers exist.

Various approaches are known for fluorescence reconstruction or localization.

Britton Chance proposed a method to localize fluorescing absorbers in homogenous medium known as phased arrays. This method localizes fluorescing inhomogeneities (spots) only in absolutely homogenous media, meaning media with homogenous light absorption properties and scatter properties (as they rarely occur in the application), and offers no information at all about the depth at which the spot is located.

Otherwise, various methods for fluorescence reconstruction have been proposed. In the reconstruction, the complete fluorescence activity in the entire

(mostly discretized) medium is determined (similar to methods in nuclear medicine), while in the localization exclusively the regions emphasized from the background are sought. Reconstruction methods thus are based on the (often iterative) solution of large equation systems and are thus, in contrast to the localization operating in real-time that is proposed here, very time-consuming. The reconstruction methods further predominantly assume that the medium (similar to computer tomography) to be examined is enclosed by a ring of light sources and detectors.

Some of the known methods are tomography with frequency-modulated light (United States Patent No. 6,304,771 and United States Patent No. 5,865,754), which requires a reconstruction time of 5 min. on a 1 GHz Pentium computer or 45 min. on a SUN Sparc 2 workstation, and tomography with light (PCT Application WO 02/41760) that likewise requires a reconstruction time of 5 min. on a 1 GHz Pentium computer.

All of these known methods are characterized by a high calculation effort and relatively small reconstruction volumes; a calculation in real-time is not possible.

SUMMARY OF THE INVENTION

An object of the present invention is to enhance the localization precision fluorescing marked tumors in a localization method of the above-described type as well as a device to implement the method, as well as to enable an evaluation in deep tissue slices, and to drastically reduce the calculation expenditure and, as a result, the calculation time.

With the inventive method, the problem of the localization of fluorescing subjects in optically blurred media can be quickly solved. Furthermore, the precision is increased via the variation of the excitation location.

In the inventive method light emission of tissue sections in which fluorescence markers are accumulated is excited by irradiation by laser light of suitable wavelength. Fluorescence light can then be measured at the proximate skin surface. In order to determine locations and optical parameters of marked tissue sections, a sequence of fluorescence excitations of the surface, for example of various locations with different modulation frequencies (including zero frequency), is radiated into the tissue, and then the fluorescent light is measured with one or more arrangements of suitable light sensors distributed on the surface, in order to thus acquire two-dimensional measurement value distributions which are dependent on the type of the excitation.

In accordance with the invention, frequency-independent signal portions are determined in the response signals, obtained by measuring the fluorescence light, and these frequency-independent signal portions are further-processed into input values for localization. The tissue section is modeled and a set of guide fields is determined from the model. The guide field are transformed, and the transformed guide fields are compared with the input values processed from the frequency-independent signal portions. A location of the transformed guide fields that best reproduces the frequency-independent signal portions is emitted, as an output, as a location of the region to be localized.

It has proven to be advantageous when, to generate the various fluorescence properties, the regions are marked with fluorescing markers (fluorophores).

The spatial resolution is enhanced when the fluorescence-exciting light signals are generated with various modulation frequencies and are irradiated into the tissue section.

It is advisable to first normalize and then transform the guide fields, whereby the guide fields can be transformed into orthogonal guide fields. Furthermore, the orthogonal guide fields can be determined from the guide fields by a singular-value decomposition.

The optical parameters can be determined in accordance with the invention by reference measurements by means of estimating methods, given non-fluorescence-exciting wavelengths.

A device for implementing the above-described method has at least one arrangement of light sensors distributed on the surface of the tissue section to measure the fluorescence light emitted by the fluorescing marked region, and laser diodes are provided to generate the light for exciting the marked region, so that a two-dimensional measurement value distribution is obtained as a result of the excitation. The output signals from the sensor arrangement are supplied to a processor for implementing the above-described method to localize the region or regions.

A measurement system can, for example, include 8x8 regular light sensors arranged on a planar measurement surface. However, it can be advantageous to measure with a number of such planar systems at the same time. Thus, for example, two arrangements of light sensors can be provided that can be applied on both sides of the tissue section to be examined. Given measurements of the female breast, two measurement surfaces can be applied to opposite sides of the mamma. An advantageous embodiment is the integrated arrangement of the measurement surfaces in pressing plates of an x-ray mammography device.

In general, any curved or curvable or flexible measurement surface can be used with any arrangement of light sensors.

DESCRIPTION OF THE DRAWINGS

Figure 1 is exposure to explain the principle of optical fluorescence imaging,

Figure 2 is an overview of the basic components of a device to localize and classify a focal lesion in a tissue section in accordance with the invention.

Figure 3 shows the substantial method steps to localize a focal lesion in accordance with the invention.

Figure 4 is a schematic illustration of an inventive applicator with 8x8 sensors as well as 8 light sources arranged near the measurement surface to generate the excitation light.

Figure 5 is a schematic illustration of a double system in accordance with the invention with two applicators positioned opposite one another.

Figure 6 is a two-dimensional measurement value distribution of a data configuration designated as the configuration 1, for the first four excitation locations.

Figure 7 shows a singular-value decomposition of the configuration 1.

Figure 8 is a basis map of the configuration 1.

Figure 9 shows localization functions of two fluorochrome-marked lesions.

Figure 10 illustrates the localization of lesions of different depths with a planar measurement system.

Figure 11 illustrates the localization of lesions of different depths with two planar measurement systems placed opposite to one another.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The overview representation in Figure 2 shows a measurement and evaluation arrangement with which a delimited spatial area 2 arranged in a biological tissue section 1 can be localized and identified. It is assumed that the spatial area 2 possesses a fluorescent property different from the remaining tissue section 1.

These assumptions are fulfilled sufficiently well when the biological tissue section 1 is a female breast and the delimited spatial area 2 is a tumor to which, for example, a fluorescing metabolic marker was supplied whose fluorescent properties are activated by specific enzymes.

The measurement arrangement includes an applicator 3 with a number of spatially distributed photo sensors, as well as additional laser diodes arranged in a line.

The recognition of a tumor or another marked region ensues by exposure of the region with light of the laser diodes in the specific excitation wavelength of the fluorescent dye, and detection of the emitted light by the photo sensors in the corresponding wavelength of the fluorophore.

The photo sensors and laser diodes of the applicator 3 are connected via electrical connection lines 4 with an electrical control device 4, and with a measurement value processor 7 via electrical connection lines 6.

With the control device 5, pulses of NIR light are supplied to the biological tissue section 1 via a number (K) laser diodes, whereby $1 \leq K \leq M$, in order to excite to fluorescence an existing marked tumor.

To localize and identify spatially delimited areas 2, the light emitted from the areas 2 is measured by photo sensors on the surface of the tissue section 1 at M locations and supplied to the processor 7.

The measurement value processor 7 includes, for example, measurement amplifiers, filters and A/D converters. The measurement value processor 7 is connected with one or more data inputs of an electronic computer 8. In addition to the measurement values, a model 9 of the tissue section 1 is available to the computer, with which the above-cited fluorescing areas 2 are localized and identified.

as is specified further below. The result, for example in the form of a graphical representation of the anatomy of the tissue section wherein the location of the light sources (and thus of the spatial areas 2) is marked, ensues via a monitor 10. Since the calculation, among other things, is determined by the model 9 and the location of the exposure, a supervisory input and control 11 is provided with which the number and the location of the photo sensors are determined, as well as the number and location of the laser diodes, the value of the frequency, and the model.

The localization method is explained using Figure 3 as an example. Explained first are its input dimensions, meaning the measurement data and model data, and then the calculation steps of the method.

The input dimensions for the localization method are, per measurement surface,

a) An *MxN* data matrix *D* with measurement values (reference number 21) which are dependent on the *M* sensor locations $\vec{r}_{S,m},(m=1,...,M)$ and the *N* excitation parameters (N_1 excitation locations $\vec{r}_{A,n_1},(n_1=1,...,N_1)$ and/or N_2 excitation modulation frequencies $f_{n_2},(n_2=1,...,N_2)$, whereby $N=N_1+N_2$), and which can result from the actual measurement data by post-processing.
The *M*-dimensional column vectors of the data matrix can be reformatted corresponding to the arrangement of the sensors on the measurement surface. A graphical representation of the reformed column vector provides a visualization of the measurement value distribution over the considered measurement surface for a given excitation type. In the case of the above-cited 8x8 sensor distribution, the 64-dimensional column vector is reformed into an 8x8 matrix.

b) A set of K guide fields or lead fields $L_k(\vec{r}_m, \vec{n}_m, \vec{r}_l, \mu_a, \mu_s), (k = 1,...,K)$, for example multipole lead fields which are characterized with the reference number 22 in Figure 3, and which for their part are dependent

- the model of the optical medium of the examination area 1,
- the measurement system, for example location $\vec{r}_m$ and/or normal vector $\vec{n}_m$ of the m-th sensor,
- the location $\vec{r}_f$ of the f-th excitable fluorochrome,
- the type of the measurement (frequency modulation yes/no) and
- optical parameters such as the absorption and scatter coefficients μ_a, μ_s of the medium surrounding the lesion(s).

The photons impacting on a sensor are transduced (converted) into electrical signals and then supplied to the further evaluation. In the case of frequency-modulated excitation, light intensity and phase shifts with regard to the input wave are measured. Both real measurement values can be combined into a complex measurement value. The data matrix is then – in the mathematical sense – complex. In the following, in the general case a complex data matrix is assumed.

It may be necessary to supply post-processed measurement data to the localization algorithm. For example, edge artifacts are eliminated via truncation of edge data. They could simulate a nonexistent dependency on the modulation frequency location or the excitation frequency.

The data matrix can result from a linear combination of at least two data sets. For example, the difference of a data set with fluorescence signals and a spatially adjacent data set without fluorescence signal can be considered. It is to be expected that possible contributions of background excitations are reduced in the difference data, if not completely eliminated.

Guide fields, known as lead fields, are known quantities from bioelectric magnetism. They describe the measurement value distribution of a standard signal source that can be acquired with a given measurement system.

Lead fields, which specify the light intensity that can be acquired with a measurement system or a number of measurement systems based on optically excited focal lesions marked with fluorochromes, are suitable as input quantities for the method to localize such focal lesions.

For example, in the exemplary embodiment only a lead field is used. It describes the light intensity of a punctiform light source measurable with a given measurement system. Corresponding to the expanded electrically-polarized lesion areas addressed in B. Scholz, "Towards Virtual Electrical Breast Biopsy: Space-Frequency MUSIC for Trans-Admittance Data", IEEE Trans. Med. Imag., Vol. 21, No. 6, pp. 588-595, spatially expanded fluorescence sources can likewise be acquired by multipole lead fields. In the following, it is assumed that exemplary lead fields, meaning a set of a number of lead fields, are available.

For the further steps, it is helpful to combine the values of the k-th lead field $L_k(k=1,...,K)$ at the M measurement locations into an M-dimensional vector in data space (symbolized by the underline under L).

(1) $$\underline{L}_k(\vec{r}) = (L_k(\vec{r},\vec{r}_1),...,L_k(\vec{r},\vec{r}_M))^T \qquad \text{with} \quad k=1,...,K$$

wherein $\vec{r}$ is the focal point of the lesion. For clarity, in equation (1) the dependency on the optical parameters of the medium surrounding the lesion(s) is not specified.

The optical parameters that, as noted above, enter into the lead fields, can be determined by reference measurements by means of estimation methods, given non-fluorescence-exciting wavelengths.

The signal processing of the method involves per measurement surface

1. the singular-value decomposition of the data matrix *D* (reference number 23 in Figure 3),
2. the analysis of the singular-value decomposition (reference number 24 in Figure 3), and
3. the actual localization method (reference number 25 in Figure 3).

The singular-value decomposition 28 of a matrix is a known mathematical method from G. Golub, Ch. Van Loan, *Matrix Computations*, 3rd edition, J. Hopkins University Press, 1996, Page 70 *et seq.*. For the above data matrix, the singular-value decomposition is

(2) $$D = U S V^H$$

wherein

- *U* a unitary *MxM* matrix dependent only on the indices of the sensor locations,
- *S* the *MxN* singular value matrix with *min(M,N)* real singular values in the diagonal and otherwise vanishing elements and
- *V* a unitary *NxN* matrix dependent only on the excitation location indices or, respectively, frequency indices and
- *H* the hermetic conjugation of the appertaining matrix.

The singular values are ordered corresponding to their decreasing numerical value, meaning

(3) $$s_1 \geq s_2 \geq \ldots \geq s_{\min(M,N)}$$

If the *q*-th column vectors of the matrixes *U* and *V* are designated by $\underline{u}_q, \underline{v}_q$, then the alternative tensorial notation (⊗ designates the tensor product)

(4) $$D = \sum_{q=1}^{\min(M,N} s_q \underline{u}_q \otimes \underline{\underline{v}}_{=q}^{H}$$

clearly shows that the *q*-th singular value is exclusively linked with the *q*-th column vectors of *U* and *V*. The single and the double underline in *u* and *v* should indicate that it concerns an *M*- or, respectively, *N*-dimensional vector.

The *M* indices of the column vectors $\underline{u}_q$ correspond to the successively numbered indices of the measurement sensors. As a result, these column vectors– as noted above – can be reformed in matrices corresponding to the arrangement of the measurement sensors and represented as two-dimensional measurement value distributions. These column vectors are excitation-independent or frequency-independent orthonormalized basis vectors in M-dimensional data space and are here designated as basis maps or eigenmaps.

For singular value analysis, the number Q_{dom} of the significant singular values is determined that specifies the number of the acting fluorescence sources linearly independent with regard to the excitation type.

A punctiform inhomogeneity in the otherwise homogenous optical medium generates, for example, a singular value spectrum with a significant singular value $(Q_{dom} = 1)$.

The associated column vectors $\underline{u}_q$ are considered as basis vectors of a – frequency-independent – Q_{dom}-dimensional signal space in M-dimensional data space. The remaining M-Q_{dom} column vectors are then the basis vector of the orthogonal signal space.

The identification of fluorochrome-marked lesions, i.e., the localization, corresponds to the search for locations or focal point locations of excited signal

sources. This search by means of computers requires the subdividing (rastering) of the adopted model medium, which should mathematically reproduce the body region to be examined.

One search strategy is to generate, at each raster location, excitation-independent and frequency-independent model data and/or a model data space with the excitation-independent and frequency-independent lead fields, and to compare this and/or these with the excitation-independent and frequency-independent signal space acquired from the measurement data. Comparison measures can be defined such that they display the degree of the "agreement" between signal space and model data / model data space. Locations at which the measure reaches a local maximum are viewed as locations of actual signal sources.

An alternative second search strategy exists in the comparison between the orthogonal signal domain – also called noise domain in the older literature – and the model data or the model data domain. Comparison measures can then be defined such that they display the degree of the "non-agreement" between the orthogonal signal space and model data / model data domain. Locations at which the measure reaches a local minimum are considered to be locations of actual signal sources.

The model data are given by the lead fields: they are either used directly or post-processed.

An individual lead field represents a model data set that reflects a specific property of the signal source. For example, the lead field of a punctiform fluorescence source describes the measurable light intensity given isotropic light emission by this source.

The entirety of the considered lead fields (number: K) defines, due to its linear independence, a K-dimensional model data space. In other words, the lead fields

are non-orthogonal basis vectors of this model data space. Orthogonal basis vectors can be acquired by suitable orthogonalization methods, i.e. by post-processing of the lead fields. They do not change the model data domain. However, new individual model data sets result with the new basis vectors (see above). These basis vectors can be additionally normalized. This ensures that lead fields with different separation behavior can be accounted for in the same manner for localization. In addition, it has the advantage of considering physically dimensionless quantities.

An advantageous lead field post-processing is, for example, to normalize the K lead fields $\underline{L}_k(k=1,...,K)$ from equation (1) (processing step 27). The individual guide fields are respectively referenced to their normalization, such that the normalized guide fields $\underline{L}_k^{(n)}$ result as follows:

(4a) $$\underline{L}_k^{(n)} = \frac{\underline{L}_k}{\|\underline{L}_k\|}$$

For example, by means of a singular-value decomposition of the M x K lead field matrix L, orthogonalized lead fields are acquired. The normalization is displayed by the index *(n)*.

(5) $$L^{(n)} = \left(\underline{L}_1^{(n)},...,\underline{L}_K^{(n)}\right) = U_L S_L V_L^T$$

For clarity, the arguments of the lead fields (the spatial vectors of the source location) have been omitted. The first K column vectors $\underline{U}(\vec{r})_{Lk}, (k=1,...,K)$ of the matrix U_L are the desired source location-dependent orthonormalized lead fields. In the case of a single lead field, the singular-value decomposition is omitted from equation (5).

For example, for the comparison measure a model data set or the model data domain and the signal or the orthogonal signal domain are known from other biomedical applications, analysis of biometric data, or analysis of electrical trans-admittance data. Such methods are projection methods and angular separation methods.

With the aid of projection matrices, individual model data sets or the model data domain are projected either on the signal space or, respectively, on the orthogonal signal domain, and determined for each raster location of the corresponding projection value.

Based on the algorithm to calculate angles between two sub-spaces, a technique known as the angular method (specified in G. Golub et al., page 584 *et seq.*, the angle between the signal domain or the orthogonal signal domain and individual model data sets or the model data domain are calculated search location by search location. Here, a small angle (thus a small value of the comparison measure) between, for example the signal domain and the model data domain, gives a large "agreement". A transformation of the comparison measure in the form of a 90° angle then again yields maxima of the comparison function at the location of the actual signal sources. The statements can be correspondingly transferred to angular comparison measures between other sub-spaces.

At each location $\bar{r}$ of the discrete optical model medium, it is tested how large the separation is between the orthogonalized lead field $\underline{U}(\bar{r})_{L,k}$ and the signal space. A suitable measure is the function

(6) $$F_k(\bar{r}) = \left[\sum_{i=1}^{Q_{dom}} c_i \underline{u}_i - \underline{U}_{L,k}\right]^2 .$$

The output equation of (6) is the equation to be considered in the sense of the quadratic mean

(7) $$\sum_{i=1}^{Q_{dom}} c_i \underline{u}_i = \underline{U}_{L,k} \qquad k = 1,\ldots,K .$$

If the solution for the coefficients c_i is used in the evaluation measure, then

(8) $$F_k(\vec{r}) = 1 - \left[\sum_{i=1}^{Q_{dom}} \left(\underline{u}_i^H \cdot \underline{U}(\vec{r})_{L,k}\right)\right]^2 .$$

This measure corresponds to a projection of the considered lead field on the orthogonal signal domain. Using the projection matrix

(9) $$P_{OS} = 1 - \sum \underline{u}_i \otimes \underline{u}_i^H$$

projected on the orthogonal signal domain results in

(10) $$F_k(\vec{r}) = \left|P_{OS}\underline{U}(\vec{r})_{L,k}\right|^2 .$$

The actual localization function F is the minimal value of the separations F_k. It is defined by

(11) $$F(\vec{r}) = \min_k \{F_k(\vec{r})\}$$

The local minima of the localization function are monotonically ordered in ascending order corresponding to their number values. The locations, which are to be associated with the first Q_{dom} local minima, are considered as locations of signal generators.

In the case of a number (M_{sys}) of measurement surfaces, the above-cited calculation steps for the data of each measurement surface are executed separately. An objective function then results per measurement surface according to equation (11). From these individual objective functions, an overall objective function $F^{(overall)}$ can be defined according to

(12) $$F^{(overall)}(\vec{r}) = \sum_{\mu=1}^{M_{sys}} F^{(\mu)}(\vec{r})$$

wherein $F^{(\mu)}$ is the objective function of the μ-th measurement surface.

The local minima of the overall localization function are monotonically ordered as above, in ascending order corresponding to their number values. The locations, which are to be associated with the first Q_{dom} local minima, are considered as locations of signal sources. The exemplary embodiment confirms the expectation that, given a plurality of non-trivial arranged measurement surfaces, the local minima of the individual objective functions are clearly formed, and thus make the localization result most reliable.

The exemplary embodiments were acquired with planar measurement systems arranged in the applicator 3, which has 8x8 regularly arranged photo sensors 31 as schematically shown in Figure 4. The sensors 31 were assumed to be punctiform. Their separation along a direction is 8 mm, such that a measurement field surface of 56x56 mm² results. The locations at which 8 laser diodes 32 which radiate the NIR light exciting fluorescence in the body region are located can, for example, be arranged near the measurement surface. The excitation can be, but does not have to be frequency-modulated. Such a measurement arrangement can be guided by hand over a tissue section 1 of interest. The laser diodes 32 emit

excitation rays 33 that impinge upon the fluorescing spatial area 2. The fluorescence rays 34 are acquired by the photo sensors 31.

In Figure 5, a double system of an applicator 3 is shown, with two planar measurement surfaces of the same dimensions (8x8 sensors) arranged opposite one another. For example, they can be integrated into the pressing plate of an x-ray mammography device. The fluorescence excitation ensues at 8 excitation locations that are located near the measurement surface (z=0) of the upper applicator 3. The separation of the two applicators 3 is 64 mm.

As an optical tissue model, in the present invention the following models are used:

A) The simplest model is a borderless area with punctiform fluorescing subjects, which is otherwise optically homogenous (constant optical parameters such as absorption coefficient and scatter coefficient).

B) As a second model, an optically inhomogeneous cuboid area with punctiform fluorescing subjects was considered. It was assumed that absorption coefficient and scatter coefficient can vary locally by 100%. Figure 9 shows the localization functions of 32 mm and 48 mm deep, fluorochrome-marked lesions. The absorption contrast difference of the surrounding tissue is 100% (image in image).

The simulation of the data is based on the following configurations:

Configuration 1

Measurement/excitation system:	see Figure 4, the excitation is not frequency-modulated
Tissue model:	inhomogeneous cuboid (5.2.B)
Fluorescence source:	location at (x,y,z)=(28,28,32) mm, meaning central position beneath the measurement surface at a depth of

Data:	32 mm (coordinate system see Figure 4) see Figure 6
Configuration 2	
Measurement/excitation system:	see Figure 4, the excitation is not frequency-modulated
Tissue model:	inhomogeneous cuboid (5.2.B)
Fluorescence source:	location at (x,y,z)=(28,28,48) mm, meaning central position beneath the measurement surface at a depth of 48 mm (coordinate system see Figure 4)
Configuration 3a, 4a and 5a	
Measurement/excitation system:	individual measurement system, see Figure 4, the excitation is not frequency-modulated
Tissue model:	homogenous, unbordered medium (5.2.A)
Individual fluorescence sources:	locations at (x,y,z)=(28,28,16) mm, (28,28,32) mm, (28,28,48) mm, meaning central positions beneath the measurement surface at depths of 16 mm, 32 mm and 48 mm (coordinate system see Figure 4)
Configuration 3b, 4b and 5b	
Measurement/excitation system:	double measurement system, see Figure 5, the excitation is not frequency-modulated

Tissue model:	homogenous, unbordered medium (5.2.A)
Individual fluorescence sources:	locations at (x,y,z)=(28,28,16) mm, (28,28,32) mm, (28,28,48) mm, meaning central positions beneath the measurement surface at depths of 16 mm, 32 mm and 48 mm (coordinate system see Figure 5)

Due to the singular-value decomposition 23 (corresponding to the number of the existing fluorescence sources), the singular value spectrum shown in Figure 7 of the data of the configuration 1 comprises a numerically dominant singular value. The remaining singular values reproduce noise, in this case numeric noise.

Figure 8 shows the associated basis maps or eigenmaps. There is a structured basis map corresponding to the single numerically dominant singular value. Here it defines the one-dimensional signal domain of the (here 64-dimensional) data space.

The above-defined objective functions for localization 25, meaning the localization functions, the configurations 1 and 2, are shown in Figure 9.

The influence of a second, oppositely-placed measurement surface on the localization is shown using the objective functions of the configurations 3 a/b, 4 a/b, 5 a/b, whereby Figure 10 shows the localization of lesions of different depths with a planar measurement system, and Figure 11 shows the localization of lesions of different depths with two planar measurement systems lying opposite one another. The positions of the measurement probes are marked by thick lines at the left edge or, respectively, at both sideways edges. With reference to Figure 10, a clearer specification of the minima is visible. It should be noted that the scale according to Figure 11 is different from that of Figure 10.

The problem of the localization of fluorescing subjects in optically bleary media can be rapidly solved with the inventive method. Furthermore, the precision is increased by the variation of the excitation location.

This inventive localization method operates in real time, is patient-independent, and is robust with regard to estimation of optical parameters.

Although modifications and changes may be suggested by those skilled in the art, it is the intention of the inventors to embody within the patent warranted hereon all changes and modifications as reasonably and properly come within the scope of their contribution to the art.